Asia-Pacific Journal of Science and Technology: Volume: 27. Issue: 02. Article ID.: APST-27-02-03. Research Article

APST

Asia-Pacific Journal of Science and Technology

https://www.tci-thaijo.org/index.php/APST/index

Published by the Research and Graduate Studies,
Khon Kaen University, Thailand

Enhanced antioxidant activity of pericarp extract from black *Dimocarpus longan* Lour.

Preaploy Hong-in[1], Chanun Punyoyai[2], Suwannee Sriyab[2], Waranya Neimkhum[3] and Wantida Chaiyana[2,*]

[1]Master of Science in Cosmetic Science, Faculty of Pharmacy, Chiang Mai University, Chiang Mai, Thailand
[2]Department of Pharmaceutical Sciences, Faculty of Pharmacy, Chiang Mai University, Chiang Mai, Thailand
[3]Department of Pharmaceutical Technology, Faculty of Pharmaceutical Sciences, Huachiew Chalermprakiet University, Samutprakarn, Thailand
[*]Corresponding author: wantida.chaiyana@gmail.com

Received 30 June 2021
Revised 23 August 2021
Accepted 30 August 2021

Abstract

Dimocarpus longan Lour. is a commercial plant that is widely grown in Asia. A large amount of its pericarp is discarded as waste. The use of this pericarp would not only reduce waste but would also increase the value of this plant. Therefore, this study aimed to investigate the chemical composition and antioxidant activity of *D. longan* pericarp. Furthermore, a novel black *D. longan* was produced and compared with the traditional dried *D. longan*. Various solvents, including petroleum ether, ethyl acetate, and 95% v/v ethanol, were used for extraction. The total phenolic content was investigated with the Folin-Ciocalteu assay, and the gallic acid content was determined with high performance liquid chromatography. The antioxidant activity of the extracts was investigated using 2,2-diphenyl-1-picrylhydrazyl,2,2'-azinobis (3-ethylbenzothiazoline-6-sulfonic acid) and the ferric reducing antioxidant power assay. The results indicate that a higher greater extract content can be obtained from black *D. longan* than from dried *D. longan*. The ethyl acetate extract of black *D. longan* pericarp contained the highest total phenolic and gallic acid contents: 11.8 ± 0.0 mg and 9.4 ± 0.0 mg/g of extract, respectively. These concentrations are significantly higher than those extracted from dried *D. longan* pericarp using the same solvent ($p < 0.001$). Moreover, the antioxidant activity of *D. longan* was enhanced after being processed to form black *D. longan*. The ethyl acetate extract possessed the greatest ferric reducing power and radical scavenging activity. Therefore, we suggest that it is a potential source of natural antioxidants that could be used in cosmetic applications.

Keywords: *Dimocarpus longan*, Antioxidant, Phenolic content, Gallic acid

1. Introduction

Dimocarpus longan Lour., a subtropical fruit-tree crop from the Sapindaceae family, is native to Southern Asia. It is found in a number of countries, ranging from Myanmar to Southern China, South-Western India, Sri Lanka, Australia, and some subtropical zones of the US [1]. It is known worldwide as longan. *D. longan* production reached 3.6 million tons worldwide in 2017 [2]. The main *D. longan* producing countries are China (50% of all production), Thailand (30%), and Vietnam (15%) [2]. An oversupply of *D. longan* in the Northern part of Thailand has reduced its price, although *D. longan* has been used in various rejuvenation remedies since ancient times. Moreover, although mature *D. longan* fruit features a succulent, delicious, white aril that has gained favor as an exotic fruit in temperate regions [3], its limited shelf life has led to it being processed into numerous preserved foods, such as dried longan and longan in syrup. A large amount of *D. longan* pericarp is discarded as waste material.

In the present study, the novel black *D. longan* product was developed through the process used for black garlic production. Garlic undergoes a heating process to form black garlic, which has enhanced concentrations of sugar and other compounds with antioxidant activity [4-6]. Many chemical reactions are induced through thermal processing, such as the Maillard reaction, which causes the color to shift from white to dark brown due to the formation of various intermediate products during the reaction and the ultimate generation of melanoidins [7]. In addition to garlic, browning occurs during the heating of several kinds of foods, such as coffee, roast

meat, and bread. The inhibition of oxidation that occurs in fresh garlic is enhanced in black garlic, a type of raw garlic that has been subjected to thermal treatment at a high temperature with a high relative humidity [8]. The production of black *D. longan* could be a way to enhance the antioxidant activity of *D. longan*. Black *D. longan* extract may have enhanced antioxidant activity and could be useful for cosmetic applications.

Since the pericarp of both traditionally dried and novel black *D. longan* is not edible, it is normally discarded as waste. As a traditional medicine, *D. longan* fruits have been used to enhance memory, promote blood metabolism, relieve insomnia, prevent amnesia, relieve stomachache, and as an antidote to different poisons [9]. Recently, dried *D. longan* aril was reported to reduce cognitive impairment in mice [10], and dried *D. longan* leaves and flowers have been reported to have antioxidant and anti-inflammatory activity [11,12]. In contrast, the pericarp of this plant is rarely used and is commonly discarded as waste that costs money to eliminate.

Therefore, this study aimed to investigate the chemical composition and antioxidant activity of pericarp from novel black *D. longan* and to compare these properties with those of traditional dried *D. longan* extract to determine its potential for use in cosmetic applications. The use of pericarp will not only decrease waste but also boost the value of this plant.

2. Materials and methods

2.1 Materials

D. longan was classified by Ms. Wannaree Charoensup, a botanist at the Herbarium of Faculty of Pharmacy, Chiang Mai University, Thailand. The voucher specimen *D. longan* number 0023268 was deposited in this Herbarium. Traditional dried *D. longan* fruit and black *D. longan* fruits were obtained from the Faculty of Agro-industry, Chiang Mai University, Thailand. The pericarp, arils, and seeds from each *D. longan* fruit were separated and subjected to a size reduction process. *D. longan* materials were kept in well-closed containers at room temperature until further use.

2.2 Extraction of D. longan

D. longan materials were extracted with the maceration method. Briefly, *D. longan* materials were macerated in petroleum ether, ethyl acetate, or 95% v/v ethanol for 24 h at room temperature. The plant material to solvent weight ratio was 1:3. The extracting solvent was filtered and removed using a rotary evaporator. The yield of each extract was calculated. All extracts were stored in the refrigerator until use.

2.3 Chemical composition determination for black D. longan extracts

Determination of the total phenolic content in each *D. longan* extract was done with the Folin-Ciocalteu method, in accordance with a previous study [13]. Gallic acid was used as a standard compound to construct a calibration curve. The results are presented as gallic acid equivalents (GAE), which represents the concentration in mg of gallic acid per g of extract. All experiments were performed in triplicate.

The chemical compositions of *D. longan* extracts were determined with high performance liquid chromatography (HPLC). A reversed phase column was used as the stationary phase. A mixture of 0.05% formic acid in acetonitrile (phase A) and 0.05% formic acid aqueous solution (phase B) was used as the gradient mobile phase. The sample was eluted at a flow rate of 1.0 mL/min, with the following gradient elution program: from 0 to 8 min, 10% phase A; from 8 to 28 min, 20% phase A; from 28 to 30 min, 30% phase A; and from 30 to 35 min, 10% phase A. The sample solutions were filtered through a 0.45 μm nylon membrane filter. Gallic acid was used as a standard compound. All the experiments were performed in duplicate.

2.4 Antioxidant activity determination

The free radical scavenging effects of *D. longan* extracts were evaluated by 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay [13,14]. The scavenging activity is reported as the percentage of inhibition. This was calculated using the following equation: %inhibition = [(C-S)/C] × 100, where C is the absorbance of the solution of DPPH radical (DPPH•) without the test solution and S is the absorbance of the solution of DPPH• with the test solution. Ascorbic acid was used as a positive control. The experiments were performed in triplicate.

The free radical scavenging effects of *D. longan* extracts were evaluated by 2,2'-azinobis (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) assay [13,15]. The free radical scavenging activity is reported as the Trolox equivalent antioxidant capacity (TEAC), which represents the amount of Trolox equivalent to 1 g of each *D. longan* extract. Ascorbic acid was used as a positive control. The experiments were performed in triplicate.

The reducing properties of *D. longan* extracts were determined based on their ferric ion reducing activity [13,16]. The reducing properties are reported as the equivalent concentration (EC_1), which represents the ferric-TPTZ reducing ability equivalent to 1 g of each *D. longan* extract. Ascorbic acid was used as a positive control. The experiments were performed in triplicate.

2.5 Statistical analysis

The results of the experiment are given as means ± standard deviations. The statistical analysis involved t-tests and ANOVA, which were performed using SPSS software (SPSS Statistics 21.0, IBM Corporations, New York, NY, USA). A significant difference was indicated when $p < 0.05$.

3. Results

3.1 D. longan pericarp extracts

D. longan pericarp extracted by petroleum ether, ethyl acetate, and 95% v/v ethanol was investigated to determine its chemical composition. HPLC chromatograms of *D. longan* pericarp extracts and the standard compound are compared in Figure 1 Gallic acid was detected at a retention time of 3.7 min. The results shown that the pericarp part of black *D. longan* extract contains gallic acid.

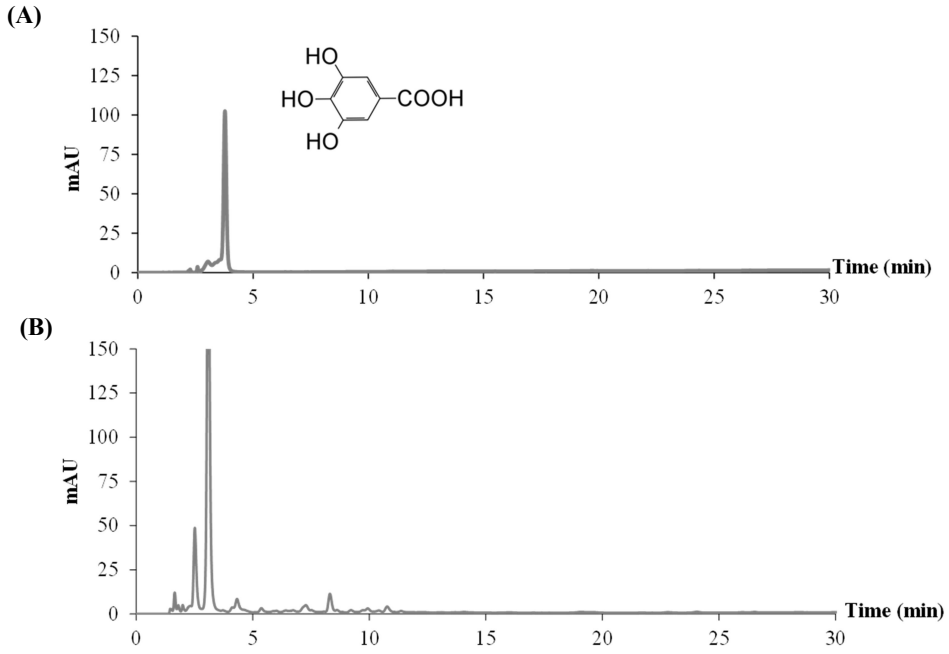

Figure 1 HPLC chromatograms of gallic acid (A) and black *D. longan* pericarp extract (B).

The chemical compositions of the *D. longan* pericarp extracts are shown in Figure 2 both the total phenolic and gallic acid contents were significantly higher in black *D. longan* pericarp extracted with ethyl acetate than in other samples ($p < 0.05$). Although no gallic acid was detected in the petroleum ether extracts of dried or black *D. longan,* small amounts of some phenolic compounds were observed. High contents of phenolic compounds were found in the ethyl acetate and ethanolic extracts of both dried and black *D. longan.* However, higher concentrations of gallic acid were observed in black *D. longan* extracted with ethyl acetate or ethanol.

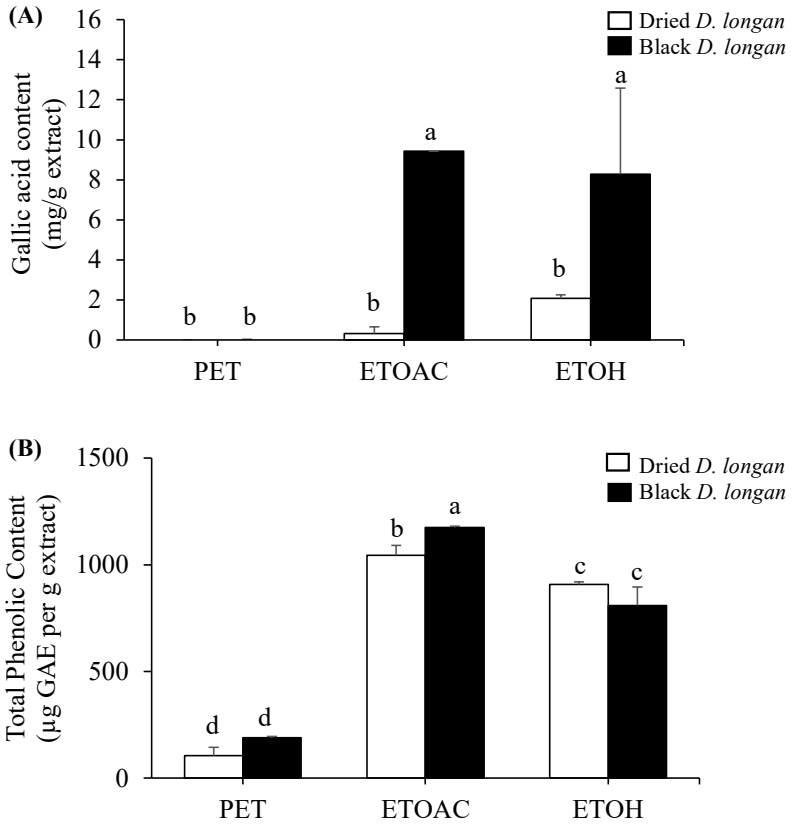

Figure 2 Gallic acid content (A) and total phenolic content (B) *D. longan* petroleum ether (PET), ethyl acetate (ETOAC), and ethanolic (ETOH) extracts. GAE = gallic acid equivalent. The letters (a, b, c and d) denote significant differences in the chemical content among *D. longan* pericarp samples extracted using different solvents ($p < 0.05$). Asterisks (*) denote significant differences between extracts.

The *D. longan* yields are shown in Figure 3 ethanolic extraction of black *D. longan* yielded the highest extract content. The amount of *D. longan* extracted was affected by both the *D. longan* materials and the solvents used in the extraction process. In dried and black *D. longan*, the same yield trend was observed, with ethanol yielding the highest extract amount, followed by ethyl acetate, and then petroleum ether. The yield of black *D. longan* was greater than that of dried *D. longan* regardless of the solvent used.

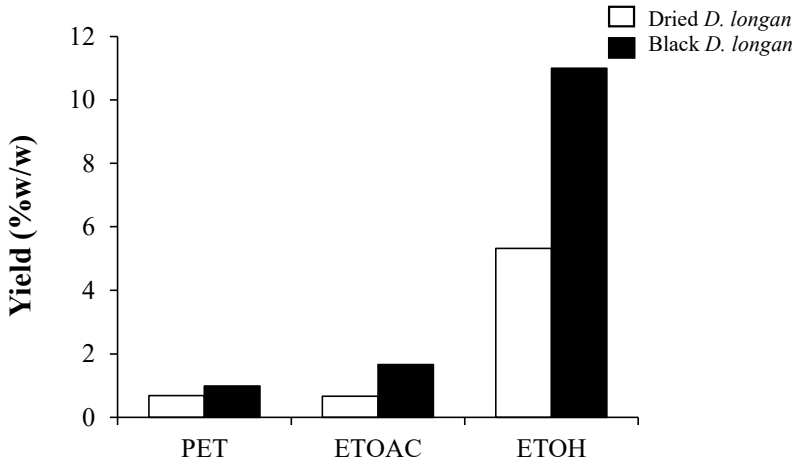

Figure 3 Yields of *D. longan* extracts obtained using petroleum ether (PET), ethyl acetate (ETOAC), and ethanolic (ETOH).

3.2 Antioxidant activity of D. longan pericarp extracts

The antioxidant activity of *D. longan* pericarp extracts is shown in Figure 4 and Figure 5. Among the various extracts, black *D. longan* pericarp extracted by ethyl acetate had the highest DPPH• inhibition and the highest TEAC and EC_1 values ($p < 0.05$). Interestingly, the TEAC value was comparable to those of ascorbic acid and gallic acid.

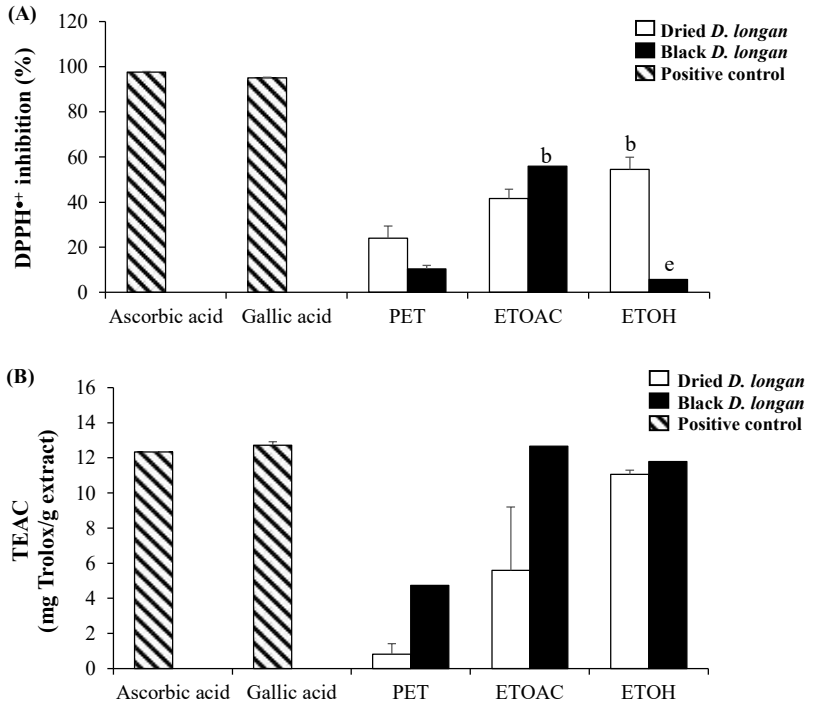

Figure 4 Radical scavenging activity of *D. longan* extract obtained with petroleum ether (PET), ethyl acetate (ETOAC), and ethanolic (ETOH). TEAC = Trolox equivalent antioxidant capacity. The letters (a, b, c, d, and e) denote significant differences between extracts ($p < 0.05$).

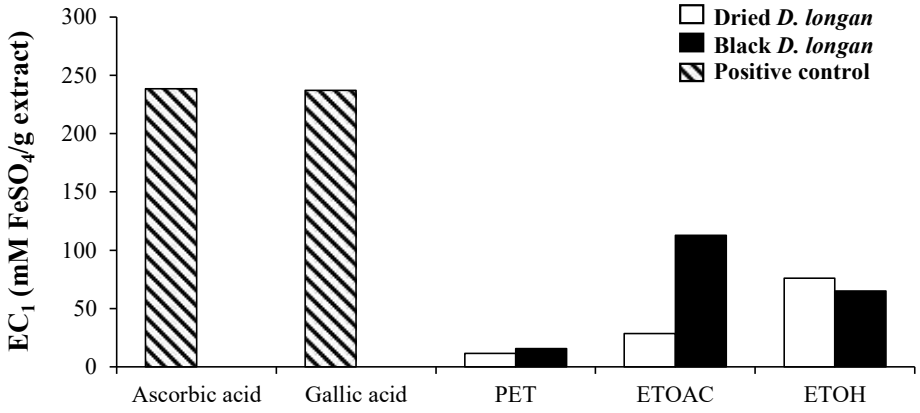

Figure 5 Ferric reducing power of *D. longan* extracts obtained with petroleum ether (PET), ethyl acetate (ETOAC), and ethanolic (ETOH). EC1 = equivalent concentration. The letters (a, b, c, d, e, and f) denote significant differences between extracts ($p < 0.05$).

4. Discussion

D. longan pericarp, which is discarded as a waste product by the food industry, contains antioxidant compounds that are beneficial to health. The amount of *D. longan* extracted and its biological activity are affected by both the *D. longan* materials and the kind of solvent used in the extraction procedure. Black *D. longan* pericarp extracted with ethyl acetate contained the highest phenolic and gallic acid contents ($p < 0.05$). Phenolic compounds, the largest class of bioactive compounds, contain one or more aromatic rings along with one or more hydroxyl groups in their backbone structure. They are secondary plant metabolites with a variety of biological roles [17]. Since phenolic compounds are mostly hydrophilic, they dissolve well in polar solvents and are efficiently extracted by hydrophilic solvents [18]. Thus, extracts obtained with ethyl acetate and ethanolic contained higher amounts of phenolic compounds than those obtained with petroleum ether. Since gallic acid was not detected in the sample extracted with petroleum ether, phenolic compounds detected in the extract could have been derivatives of gallic acid, such as 4-*O*-methylgallic acid, methyl gallate, and ethyl gallate [17,19] and other kinds of phenolic compounds, such as ellagic acid, corilagin, chebulinic acid, and epicatechin. Interestingly, a higher gallic acid content was observed in black *D. longan* extract than in dried *D. longan*. Certain mechanisms change the phenolic content of a sample when the temperature increases [20]. The release of bound phenolic compounds involved in the degradation of lignin generates the release of phenolic acid derivatives and initiates heat degradation of the phenolic compounds [21]. Accordingly, black *D. longan*, which underwent incubation under high temperature and high humidity conditions, was found to have a higher gallic acid content than dried *D. longan*. The antioxidant activity of *D. longan* pericarp extracts was in accordance with the total phenolic content. Phenolic compounds have been reported to be the most common and abundant antioxidants in a variety of medicinal plants [22]. The antioxidant activity of phenolic compounds is directly associated with the number and positions of their hydroxyl groups [23]. The phenolic nucleus enhances antioxidant activity through its hydroxyl groups. The positions of hydroxyl groups on a phenol ring as butyl/ethyl groups at para and para/ortho positions influences the antioxidant activity of phenolic compounds [24,25]. Therefore, the presence of phenolic substances in plants with antioxidant activity is related to the chemical structure and corresponds to the occurrence of phenolic constituents in plants [26]. In this experiment, it was found that gallic acid in *D. longan* extracts are indicative of scavenging activity.

Consequently, phenolic compounds are suggested to be biological constituents that are responsible for the DPPH• scavenging activity of black *D. longan* extracts. These extracts were found to have the highest radical scavenging and ferric reducing capacities ($p < 0.05$). Three antioxidant assays, DPPH, ABTS, and the FRAP assay, were used in the present study to confirm the antioxidant activity of *D. longan* extracts, because various mechanisms of oxidation should be investigated concurrently. The DPPH assay was developed to investigate the potential of compounds to scavenge free radicals or act as hydrogen donors by mixed single electron-transfer (SET) and hydrogen atom transfer (HAT) mechanisms, whereas the ABTS assay is based on HAT [27,28]. The FRAP assay is based on the reduction of ferric ions (Fe^{3+}) to ferrous ions (Fe^{2+}) [29].

Each *D. longan* pericarp extract has a different polarity, ranging from petroleum ether extract, which is lipophilic, to ethyl acetate extract, which is hydrophilic, to ethanolic extract, which is more hydrophilic. The *D. longan* ethanolic extract was found to have the highest antioxidant activity with the ABTS assay, but diminished antioxidant activity was detected in the DPPH assay because, in contrast to the DPPH assay, which is preferably run using hydrophobic organic solvents, the ABTS assay can be utilized in water-soluble systems [27,28]. However, the results from the ABTS assay were similar to those from the FRAP assay. This was likely due to the equivalent redox potential of Fe (Fe^{3+}) salt (-0.70 V) and ABTS radical ($ABTS^{\bullet+}$) (0.68 V).

In brief, gallic acid is a major bioactive component of black *D. longan* pericarp and is responsible for its antioxidant activity. Additionally, the production of black *D. longan* could enhance both the biological active content and the antioxidant activity of *D. longan*. Our results are in accordance with those of our previous study, which investigated the phenolic content and biological activity of ethanolic extracts from various parts of dried and black *D. longan* [30]. However, the present study focused on the utilization of the pericarp, since it is discarded as waste and currently has no value, whereas the arils are edible and usually firmly attached to the seeds, so they are often sold together. In the present study, various solvents were used for the extraction of biologically active compounds from both dried and black *D. longan* pericarp. Interestingly, the extract obtained with ethyl acetate presented the most promising antioxidant activity. It was found to be more potent than the extract obtained with ethanol presented in our previous study [30]. This use of pericarp would not only decrease waste but would also boost the value of this plant.

5. Conclusion

Black *D. longan* pericarp extract obtained with ethyl acetate could be a potential source of natural antioxidants that could be used in cosmetic applications. It has comparable $ABTS^{\bullet+}$ radical scavenging activity to ascorbic acid and gallic acid. The use of black *D. longan* pericarp, an agricultural waste product, would be a first step toward achieving a zero-waste state. Additionally, imported cosmetic ingredients from foreign countries could be substituted with this natural product from local plants.

6. Acknowledgements

This work was financially supported by the Agricultural Research Development Agency (ARDA), Thailand, under grant number PRP6305032210. The authors would like to acknowledge the Faculty of Agro-industry, Chiang Mai University, Thailand for providing the traditional dried and black *D. longan* materials.

7. References

[1] Zhang X, Guo S, Ho CT, Bai N. Phytochemical constituents and biological activities of longan (*Dimocarpus longan* Lour.) fruit: a review. Food Sci Hum Well. 2020;9(2):95-102.

[2] Minor tropical fruits. http://www.fao.org/fileadmin/templates/est/comm_markets_monitoring/tropical_fruits/documents/minor tropical fruits foodoutlook 1 2018.pdf; [accessed 2021 April 4].

[3] Yang B, Jiang Y, Shi J, Chen F, Ashraf M. Extraction and pharmacological properties of bioactive compounds from longan (*Dimocarpus longan* Lour.) fruit-a review. Int Food Res J. 2011;44(7):1837-1842.

[4] Shin JH, Choi DJ, Lee SJ, Kwon OC, Cha JY, Sung NJ. Antioxidant activity of black garlic (*Allium sativum*). J Korean Soc Food Sci Nutr. 2008;37:965-971.

[5] Choi DJ, Lee SJ, Kang MJ, Cho HS, Sung NJ, Shin JH. Physicochemical characteristics of black garlic (*Allium sativum*). J Korean Soc Food Sci Nutr. 2008;37:465-471.

[6] Choi IS, Cha HS, Lee YS. Physicochemical and antioxidant properties of black garlic. Molecules. 2014;19:16811-16823.

[7] Liu P, Lu X, Li N, Zheng Z, Zhao R, Tang X, et al. Effects and mechanism of free amino acids on browning in the processing of black garlic. J Sci Food Agr. 2019;99(10):4670-4676.

[8] Jing H. Black garlic: processing, composition change, and bioactivity. eFood. 2020;1(3):242-246.

[9] Longan production in Asia. http://www.fao.org/3/X6908E/x6908e00.htm; [accessed 2021 April 4].

[10] Li H, Lei T, Zhang J, Yan Y, Wang N, Song C, et al. Longan (*Dimocarpus longan* Lour.) aril ameliorates cognitive impairment in AD mice induced by combination of D-gal/AlCl3 and an irregular diet via RAS/MEK/ERK signaling pathway. J Ethnopharmacol. 2021;267:113612.

[11] Li L, Xu J, Mu Y, Han L, Liu R, Cai Y, et al. Chemical characterization and anti-hyperglycaemic effects of polyphenol enriched longan (*Dimocarpus Longan* Lour.) pericarp extracts. J Funct Foods. 2015;13: 314-322.

[12] Tandee K, Kittiwachana S, Mahatheeranont S. Antioxidant activities and volatile compounds in longan (*Dimocarpus longan* Lour.) wine produced by incorporating longan seeds. Food Chem. 2021;348:128921.

[13] Chaiyana W, Punyoyai C, Somwongin S, Leelapornpisid P, Ingkaninan K, Waranuch N, et al. Inhibition of 5α-reductase, IL-6 secretion, and oxidation process of *Equisetum debile* Roxb. ex vaucher extract as functional food and nutraceuticals ingredients. Nutrients. 2017;9(10):1-17.

[14] Brem B, Seger C, Pacher T, Hartl M, Hadacek F, Hofer O, et al. Antioxidant dehydrotocopherols as a new chemical character of *Stemona* species. Phytochem. 2004;65(19):2719-2729.

[15] Tachakittirungrod S, Okonogi S, Chowwanapoonpohn S. Study on antioxidant activity of certain plants in Thailand: mechanism of antioxidant action of guava leaf extract. Food Chem. 2007;103(2):381-388.

[16] Saeio K, Chaiyana W, Okonogi S. Antityrosinase and antioxidant activities of essential oils of edible Thai plants. Drug Discov Ther. 2011;5(3):144-149.

[17] Rakariyatham K, Zhou D, Rakariyatham N, Shahidi F. Sapindaceae (*Dimocarpus longan* and *Nephelium lappaceum*) seed and peel by-products: potential sources for phenolic compounds and use as functional ingredients in food and health applications. J Funct Foods. 2020;67:103846.

[18] Zuorro A, Lavecchia R. Influence of extraction conditions on the recovery of phenolic antioxidants from spent coffee grounds. Am J Appl Sci. 2013;10:478-486

[19] Sun J, Shi J, Jiang Y, Xue SJ, Wei X. Identification of two polyphenolic compounds with antioxidant activities in longan pericarp tissues. J Agric Food Chem. 2007;55(14):5864-5868.

[20] Maillard MN, Berset C. Evolution of antioxidant activity during kinling: role of insoluble bound phenolic acids of barley and malt. J Agric Food Chem. 1995;43:1789-1793
[21] Ross CF, Hoye Jr C, Fernandez-Plotka VC. Influence of heating on the polyphenolic content and antioxidant activity of grape seed flour. J Food Sci. 2011;76(6):884-890.
[22] Hossain MS, Uddin MS, Kabir MT, Begum MM, Koushal P, Herrera-Calderon O. *In vitro* screening for phytochemicals and antioxidant activities of *Syngonium podophyllum* l.: an incredible therapeutic plant. Biomed. Pharmacol J. 2017;10:1267-1277.
[23] Rodriguez-Bonilla P, Gandia-Herrero F, Matencio A, Garcia-Carmona F, Lopez-Nicolas JM. Comparative study of the antioxidant capacity of four stilbenes using ORAC, $ABTS^{+}$, and FRAP techniques. Food Anal Method. 2017;10:2994-3000.
[24] Rice-Evans CA, Miller NJ, Paganga G. Structure-antioxidant activity relationships of flavonoids and phenolic acids. Free Radic Bio Med. 1996;20:933-956.
[25] Heim KE, Tagliaferro AR, Bobilya DJ. Flavonoid antioxidants: chemistry, metabolism and structure-activity relationships. J Nutr Biochem. 2002;13:572-584.
[26] Panpipat W, Suttirak W, Chaijan M. Free radical scavenging activity and reducing capacity of five Southern Thai indigenous vegetable extracts. Walailak J Sci Tech. 2010;7(1):51-60.
[27] Shalaby EA, Shanab SM. Antioxidant compounds, assays of determination and mode of action. Afr J Pharm Pharmacol. 2013;7:528-539.
[28] Schaich KM, Tian X, Xie J. Hurdles and pitfalls in measuring antioxidant efficacy: a critical evaluation of ABTS, DPPH, and ORAC assays. J Funct Foods. 2015;14,111-125.
[29] Somwongin S, Chantawannakul P, Chaiyana W. Antioxidant activity and irritation property of venoms from *Apis* species. Toxicon. 2018;145:32-39.
[30] Hong-in P, Neimkhum W, Punyoyai C, Sriyab S, Chaiyana W. Enhancement of phenolics content and biological activities of longan (*Dimocarpus longan* Lour.) treated with thermal and aging process. Sci Rep. 2021;11:15977.